# Hiroshi INOUE*: Regeneration of the leaf of *Acrobolbus ciliatus*

## 井上 浩*: *Acrobolbus eiliatus* の葉の再生

It is a well known fact that the leaf cells of bryophytes have the reproductive ability, and many reports on the regeneration have been published. Those studies were summarized by Fulford (1956), recently. She concluded that the reproductive pattern of the leaf cell essentially follows that of the sporeling. In this repsect, it may forward the knowledges on sporeling pattern as well as reproduction to study the regeneration of leaf cell in those species whose sexual reproduction are unknown.

*Acrobobus ciliatus* has been known only in sterile condition, except for my short description on male plant (Inoue 1956). Therefore, it may be considered that the reproduction of this species is in asexual way by the regeneration of leaf cell or others, as noted by Schuster (1958). This way may be suspected by the fact that the leaves are more or less caducous and the marginal cells of leaves have the ability to become rhizoids. However, in natural habitat and also among herbarium materials, the regenerative plantlets of this species can be hardly found.

The present study was undertaken in order to clear the regenerative ability, the pattern of regeneration, and the pattern of rhizoid formation of this species.

The materials used for this study were collected at Ōchigawa, Chichibu, Saitama Pref., on July 21 and Nov. 3, 1957. They were cultured for about a month before the treatment. The leaves were dissected away from both newly produced shoots and old ones. The leaves were washed with distilled water three times; then they were placed on sterilized filter paper moistened by glucose solution (1%) or on glucose agar (1.5% agar in 1% glucose solution) in petri dishes. The pH of the media were adjusted to 6.0 or 7.0. The petri dishes were placed at a distance of 45 cm. from a fluorescent lamp under the laboratory temperature.

## Observation and Discussion

1) Rhizoid formation of marginal leaf cell: Rhizoids were mostly formed on the leaves dissected away from a new shoot, and the formation took place in 6

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weeks after the treatment. Stages of the rhizoid formation are shown in figs. a-c. At first, the rhizoid initial cell cannot be distinguished from other cells. By 0.1% neutral red, however, it is stained faintly reddish, while other cells are not. As the initial cell becomes large in size, the chloroplasts are dissolved and become granular, and the protoplasm is stained more reddish by 0.1% neutral red. The initial cell elongates to right angle to the leaf margin and the cell wall of elongated portion is thickened gradually. After the dissolution of the chloroplasts, the oil-bodies of the initial cell remain as they were before; but they disappear at the stage that the cell wall is thickened. This cell elongates to a marginal rhizoid.

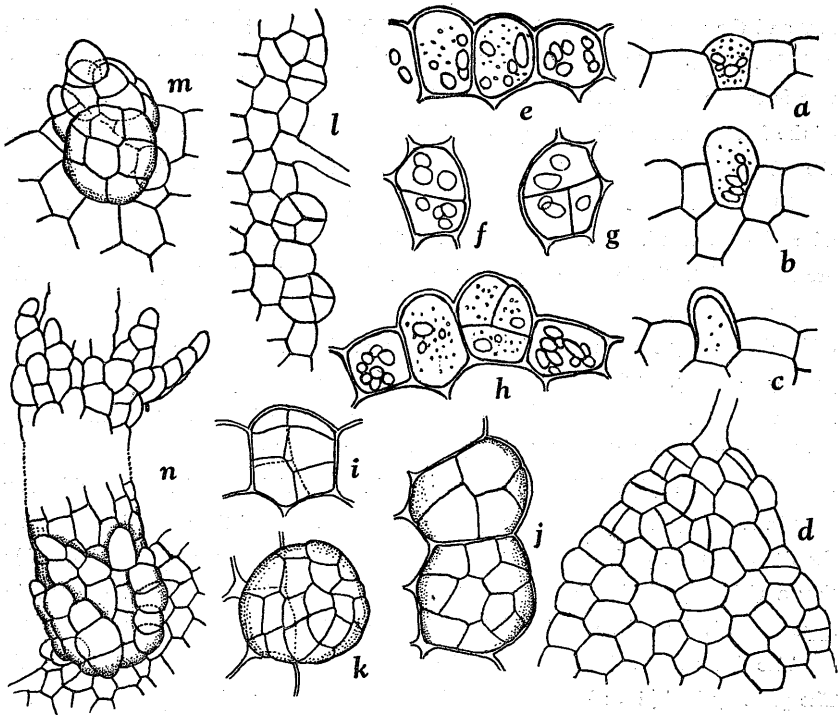
2) Regeneration from leaf cell: The first sign of the regeneration was seen after about a month from the treatment. The cells from which reproductions occur are restricted to the leaf margin and the apical part of leaf lobe (figs. d. l). At first, the cell contents become granular and the nucleus becomes conspicuous. The oil-bodies usually remain as before, but some of them become granular and disappear (figs. e, h). The first cell division takes place radially or tangentially to the leaf margin. Before this stage the cells hardly change its size. The initial cell gradually bulges and the cell divisions take place several times in the framework of the original cell (figs. i, j). The cells within the original cell may considerably vary in number, sometimes as many as 15. As a result of the successive cell divisions the cells are pushed out of the surface of leaf by the other inner cells (fig. k); thus a globose pile of cells (regenerant protonema) which resembles a gemma in appearance is formed on leaf margin and apex of leaf lobe.

The leafy shoot develops from the distal tip of globose protonema (fig. m). The primary leaf (fig. n) which arises just above the apex of globose protonema is triangular but occasionally very rudimentary. The juvenile leaf (fig. n) is distinctly bifid, and the underleaf never occurs. No rhizoids are observed in both regenerants and protonema.

At the earlier stage of the development of the protonema the oil-bodies are not formed; after the pile of cells is developed they are formed within the cells of protonema. The oil-bodies are very small (about 2$\mu$ in size) and 4–6 per cell, faintly brownish gray in color, and the inner granules are usually obscure.

From the above study, it can be said that the leaf cell has rather strong ability to regenerate. This fact may serve to conclude that the actual reproduction of this species is in asexual way by the regenerant from leaf cell, as Schuster (1958, p. 45, foot note 33) noted; "This species reproduces asexually by tardily and sporadically

caducous leaves." As eventually regenerants or gemmalings are not observed in field, there remains a question whether the above-mentioned regeneration actually occurs in field or not.




Rhizoid formation (figs. a-c) and regeneration (figs. d-n) of leaf cell of *Acrobolbus ciliatus*. For explanation see text. Figs. a-c, e-k, and m, x 360; figs. d, l, and n, x 160.

The pattern of regeneration of this species follows that of the *Nardia*-type sporeling. The rhizoids on leaf margin may have nothing to do with the regeneration of leaf cell. The presence of the ability to produce rhizoid on leaf margin, however, may indicate that the histological differentiation of leaf-margin and stem is not definitive. The rhizoid initial cell and regenerative cell cannot be distinguished from the other or from ordinary cells. But the fact that the rhizoid initial cell is stained by neutral red seems to indicate that protoplasm of the rhizoid initial cell is not the same as other cells physiologically. Therefore, it is considered that the differentiation of rhizoid initial cell and regenerative cell follows another pattern

at ealier stage. Probably, the protoplasmic change may occur in the rhizoid initial cell to show special affinity to neutral red.

## Summary

Leaf cells of *Acrobolbus cilliatus* whose sexual reproduction is unknown have strong ability to regenerate. The regenerative pattern follows the *Nardia*-type sporeling. The cells from which regeneration take place are restricted to leaf margin and apex of leaf lobe. The rhizoid initial cells which are also restricted to leaf margin cannot be distinguished from their neighbouring cells, but they have special affinity to neutral red, and by which they are stained reddish.

I am much indebted to Dr. S. Hattori and Prof. H. Ito for their kind guidances and many suggestions.

## Literature cited

Fulford, M. (1956) Phytomorph. **6** : 1–235, f. 1–236. Inoue, H. (1956) Journ. Jap. Bot. **31** : 340–344, f. 1–2. Schuster, R. M. (1958). Bryologist **61** : 1–66.

## Appendix: Taxonomic note

Japanese *Acrobolbus titibuensis* (Hatt.) Hatt. is essentially conspecific with *A. ciliatus*.

**Acrobolbus cilliatus** (Mitt.) Schiffn. Nat. Pfl. -fam. **1** (3) : 86 (1893).—*Acrobolbus rhizophyllus* Sharp, Bryologist **39** : 1 (1936)—*Leiocolea titibuensis* Hatt. Journ. Jap. Bot. **19** : 197, f. 17 (1943)—*Acrobolbus titibuensis* (Hatt.) Hatt. loc. cit. **26** : 96 (1951); Bryologist **54** : 113(1951), **syn. nov.**

Therefore, this species distibutes disjunctively as follows:

India (Sikkim-Himalaya), Japan (Kyushu, Shikoku, Honshu), and North America (N. Carolina, Tennessee).

## 摘　　要

*Acrobolbus ciliatus* はインド，日本[3]，北米に各数カ所が生育地として知られているが，従来本種の生殖器官についてはほとんど知られていない。筆者は先年秩父産の資料にもとずいて雄の植物をかんたんに記載したが，雌の植物はその後になつてもどこからも報告されていない。従つて本種が有性的な方法でふえることは先づないと考えられ

3) 日本産の *A. titibuensis* は北米，インドのものと同一種である。従来は日本，北米，インドのものは各々別種とされていたが，三種が非常に近似であることはすでに服部博士も指摘している。ごく最近 R. M. Schuster は北米のもの (*A. rhizophyllus*) をインドのものと同一種とした。

る。最近 R. M. Schuster (1958) は本種が葉の再生による無性生殖を行うことを，葉が比較的ちぎれ落ち易い性質から考えているが，今回の研究はこの Schuster の考えを支持するものであろう。すなわち葉辺の細胞および葉頂部の細胞に比較的再生力が強いことがわかつた。しかし自然状態においては本種の葉には再生的な無性芽等が発見されていないので，実際にこのような葉細胞の再生による無性的な方法が行われているかどうかはなお問題として残る[4]。

葉細胞の再生パターンは Fulford (1956) のいう *Nardia*-type である。また本種の葉辺に生ずる特殊な仮根と再生細胞との関係についてみると，仮根のない葉でも容易に再生することから，両者には直接的な関係がないといえる。仮根の原基細胞は最初は他の細胞と形態的には区別されないが，仮根分化が始まつていると neutral red で仮根原基細胞のみが染まる。染まる濃さは分化が進むほど濃くなる。一方再生細胞は分化を始めても染色されない。このようなことから仮根の原基細胞と再生細胞とは分化の初期に原形質内の生理的パターンの変化が全く異なる方向に起るものと考えられる。また葉辺の仮根は茎に生ずる仮根とその染色性その他の性質が同じであることから，茎の細胞と葉辺部の細胞との組織的分化は明確ではないということができると考えられる。

4) このことに関連したことであるが東京都の丹波村で採集した本種は茎の下部（比較的古い部分）にたくさんの小さな側枝を出しているものが多かつた。このような現象（これは茎からの再生と考えられる）も無性的なふえ方として有利な方法であろう。

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**○アゼスゲ節について**（秋山茂雄）　Shigeo AKIYAMA: Adequate name for sect. Acutae of *Carex*.

本節は *Carex acuta* L. をふくみ，Section 名には古来 Acutae が用いられていた。それで筆者が "極東亜産スゲ属植物 1955" の原稿をかきだしたのは15年以上昔であつたので，つい Sect. Acutae を用いてしまつたのであるが，命名規約によりこれは Sect. Carex とすべきものであるので下記のように訂正させていただきます。

*Carex* (Typus: *Carex acuta* L.) Sect. **Carex**

Sect. Acutae Fries, Corp. Fl. Suec. 191 (1835).

Sect. 25. Acutae Akiyama, Car. Far East Reg. Asia: 71 (1955).

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**□吉川純幹：日本スゲ属植物図譜** (II) A5 pp. 139 図版 70

本書は本誌33巻2号で大井博士が紹介された第1巻の続巻で，タニガワスゲ他70種を図説し，図版の対照頁が説明になっている。すべて前記大井氏の紹介の通りである。いま両巻をあわせると140種のスゲが見られることになり更に続巻を合せば日本のスゲの大部分がわかることになるので続巻刊行のため更に努力するよう著者と発行者に期待する。

J. Yoshikawa: **Icones of Japanese Carex,** Vol.**2**. 139 pp. incl. 70 pls. (1958) Publ. by Hokuriku-no-Shokubutsu-no-Kai, c/o Botanical Institute, Faculty of Science, Kanazawa Japan.　（久内清孝）